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Lippia spp. Essential Oil as a Control Agent against *Acanthoscelides obtectus*, an Insect Pest in *Phaseolus vulgaris* Beans †

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Abstract: *Acanthoscelides obtectus* Say (Coleoptera: Chrysomelidae: Bruchinae), know as the bean weevil, causes severe post-harvest losses in common bean (*Phaseolus vulgaris* L.). The control of this insect pest is still poor and involves the use of conventional insecticides. Besides, there is an increasing demand in the search for new active substances and natural plant products for pest control towards the reduction of adverse effects on human health and the environment. Under laboratory conditions, four doses (12, 24, 60 and 120 μL) of *Lippia origanoides* and *Lippia alba* essential oils were evaluated on Petri dishes against *A. obtectus* insects during 15 days. *L. origanoides* and *L. alba* oils exhibited similar patterns of insecticidal activity over the insect. *L. origanoides* and *L. alba* accumulated an insect mortality of 85.00 and 81.94% at dose 120 μL, respectively, significantly greater than the lower applied doses of each essential oil. However, all the lower doses applied of each oil produced significantly higher effects than the control treatment, with an accumulated mortality of 16.25%. These essential oils affected the survival of *A. obtectus* since the greatest doses applied on insects decreased the life span of the bean weevil. The results prove the insecticidal capacity of the essential oils of *Lippia* spp. genus and hence their potential as active substances against *A. obtectus* in environmentally low-risk pest control strategies. Supplementary trials should be conducted under real storage conditions.

Keywords: essential oils; stored bean pest; *Lippia alba*; *Lippia origanoides*; Insecticidal activity

1. Introduction

Acanthoscelides obtectus Say (Coleoptera: Chrysomelidae: Bruchinae), know as the bean weevil, is a pest that thrives primarily in stored common beans *Phaseolus vulgaris* L. (wild and cultivated) [1–3]. *A. obtectus* attacks *P. vulgaris* seeds while they are still in the field, and the damage continues during storage, where it causes the greatest losses [4]. When *P. vulgaris* beans are no not treated, *A. obtectus* population grows exponentially causing the loss of whole crops within a very short time [5].

The control and management of *A. obtectus* during the last years in big storage has been through the use of chemistry synthesis products, such as phosphine, pyrethroids and organophosphates [6]. These products are highly toxic to human health and environmental, to which is added the problem of being able to develop resistances on the part of the insects against these products [7] for continued use. New lines of research for pest control focus on the development of new compounds with greater selectivity, less environmental persistence, different modes of action and new sustainable alternatives [8,9]. Plant metabolites and essential oils possess these characteristics and pose substantially fewer risks than those of traditional chemical insecticides. Natural plant products have been proposed worldwide as an alternative for the control of mites and insect pests [10–15]. Studies have shown the insecticidal potential of essential oils and their capacity to disrupt insect development [16,17] through the interference over the insect nervous system, as for example, andaminergic transmissions [18–21] or the inhibition of acetylcholinesterases [22–24].

L. origanoides H.B.K. (Verbenaceae), is an aromatic plant native from Central and northern South America [25]. At domestic level, this aromatic plant is used for food sea-soning but is also widely used in folk medicine [26,27]. Part of the health benefits of this plant is attributed to the essential oil of this plant [28].

L. alba is one of the most important medicinal plant used by Latin American people [29]. Among the beneficial properties of *L. alba* described, can highlight antifungal, antimicro-bial and antioxidant properties [30–32]. That is, the health benefits reported and bioactive properties of this plant are directly associated to the composition of the essential oils of *L. alba* [33].

The present study explores the insecticidal potential of *L. origanoides* and *L. alba* essential oils against *A. obtectus* on stored beans by non-fumigant applications.

2. Experiments

2.1. Insects Rearing

The original population of *A. obtectus* adults was collected in different bean storage facilities, all of them located in the Protected Geographical Indication (PGI) "Alubia de La Bañeza-León" that certifies the quality and high standards of beans from this region, (EC Reg. n.256/2010 published on 26 March 2010, OJEU L880/17). The insects were reared in glass jars (150 mm in diameter and 250 mm high) with common bean (*P. vulgaris*) seeds and covered with cloth, allowing the gas exchange. Every three days all *A. obtectus* adults were removed from the jar in order to maintain a population of young adults (1 to 3-day old) ready to use in the experiments. *A. obtectus* adults, before and after the treatments, were kept in a chamber with controlled temperature (24 ± 1° C), humidity (60 ± 5%), and a photoperiod of 16 h of light (luminous intensity of 1000 lux) and 8 h of darkness.

2.2. Essential Oils

L. origanoides and *L. alba* essential oils obtained by hydrodestillation and, volatile components of essential oils were identified by Gas Chromatography with Flame Ionization Detection (GC/FID) and Gas Chromatography with Mass Spectrometry Detection (GC/MS)[33,34]. Analysis for the main compounds are depicted in Table 1.

2.3. Design of Experiments

Experiment 1: Essential Oil Effects on *A. obtectus* Adults

This bioassay was conducted to determine the dose-dependent toxicity of *L. origanoides* and *L. alba* essential oils against *A. obtectus* adults. For the treatments application, a Potter Tower (Burkard Scientific Limited, Po Box 55 Uxbridge, Middx UB8 2RT, U.K.) [35] of manual loading coupled to an air compressor was used. The total volume used in each spray was 1 mL, applied on Petri dishes (90 mm in diameter) covered with a sterile filter paper (Sigma-Aldrich Chemie GmbH, Steinheim, Germany), to make sure that the treatments were retained, at 40 kPa. Four doses (12, 24, 60 and 120 μL/petri dish) of *L. origanoides* and *L. alba* were diluted in ethanol and four replicates were performed for each of them. A treatment with ethanol (without oil) was used as a control. After application of treatments, twenty unsexed 1 to 3-day old *A. obtectus* were placed in the Petri dish. From now, 'Petri dish' will be considered as the basic test unit. The Petri dishes were kept in a chamber with controlled temperature (24 ± 1° C), humidity (60 ± 5%), and a photoperiod of 16 h of light (luminous intensity of 1000 lux) and 8 h of darkness. In the covers of Petri dishes, 8 holes of 1 mm diameter (8 mm^2) were made to avoid the vapour accumulation effect from the treatments. Daily monitoring was carried out during the following 15 days after the application of each dose of essential oil, counting the mortality of *A. obtectus* adults.

2.4. Statistical Analysis

Experiment 1. A randomly completed experiment Generalized Linear Model (GLM) procedure, with four doses for each essential oil and four replicates, was subjected to ANOVA (data means were normally distributed and presented homocedasticity). Differences ($p < 0.05$) on the same day among doses (within the same essential oil), and the control, were examined by mean comparisons using the Least Significant Difference (LSD) test. The mortality data were corrected with the Abbott's formula [36] in the experiment described. Mean values and standard errors are given in Figure 1 (Mortality of *A. obtectus* on Petri dishes).

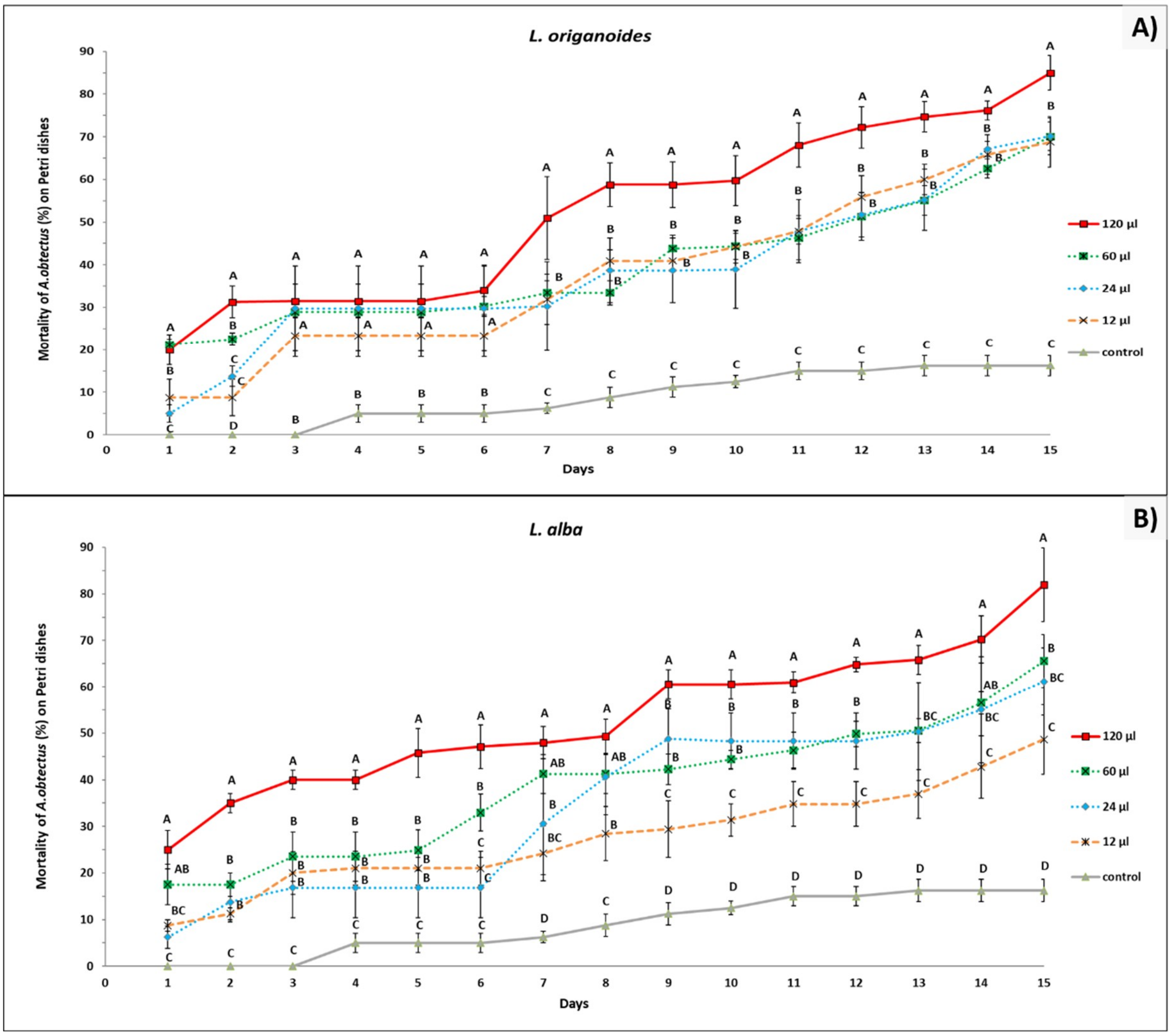

Figure 1. Accumulated mortality of *A. obtectus* on Petri dishes exposed to different doses of *L. origanoides* (**A**) and *L. alba* (**B**) essential oils. Different capital letters indicate significant differences among doses and control for the same day; LSD test at 0.05. The symbols represent the mean of four replicates for each dose. Vertical bars represent the Standard Error (SE) of the mean.

Table 1. Composition of *L. origanoides* and *L. alba* essentials oils obtained with gas chromatographic analysis.

Essential Oil	Oil Compounds (%)	Essential Oil	Oil Compounds (%)
L. origanoides	p-Cymene (6.27) γ-Terpinene (3.97) Linalool (2.53) Camphor (1.10) Thymol methyl ether (2.53) Thymol (4.10) Carvacrol (58.6) β-Caryophyllene (4.13) α-Humulene (1.43) Bicyclogermacrene (3.23) Spathulenol (2.20) Caryophyllene oxide (1.60)	*L. alba*	1-Octen-3-ol (1.80) β-Myrcene (12.00) Limonene (9.70) Neral (10.50) Carvone (25.17) Geranial (11.87) Caryophyllene (3.53) Germacrene D (8.73)
Total identified (%)	(91.69)	Total identified (%)	(83.30)

3. Results

3.1. Mortality of A. obtectus Adults against Different Doses of Essential Oils (Experiment 1)

Figures 1A,B show the significant differences among doses of essentials oils when they were applied directly on *A. obtectus* adults placed in Petri dishes.

For *L. origanoides* essential oil, the best control was achieved for the dose 120 μL on the day 15 after aplication, killing 85.00 ± 4.07% of the adults evaluated. This value was significantly greater (F = 14.411; df = 4.15; $p < 0.001$) than the obtained for the other doses tested. The doses 60, 24 and 12 μL were able to kill 70.07, 70.16 and 68.79% of adults evaluated, respectvely. The control treatment significantly differed (F = 11.287; df = 4.15; $p < 0.001$) from the 1st day after application, in which percentage of mortality was 16.25 ± 2.39% on day 15th (Figure 1A).

Likewise, the best control capacity obtained for *L. alba* essential oil was achieved for the dose 120 μL on day 15 after aplication, killing 81.94 ± 7.91% of the adults evaluated. This value was significantly greater (F = 40.333; df = 4.15; $p < 0.001$) than for the other doses. Among the lowest doses, the dose μL, with 65.55 ± 5.75% of the dead insects, also was significantly higher than the dose 12 μL, with 48.65 ± 7.47% of the dead insects. Again, the control treatment significantly differed (F = 11.287; df = 4.15; $p < 0.001$) from the 1st day after application, in which percentage of mortality was 16.25 ± 2.39% on day 15th (Figure 1B).

4. Discussion

All the insecticidal properties of essential oils are based on the action of their different components that make them up.

The essential oil of *L. origanoides* was principally composed by the monoterpenoid phenol Carvacrol (58.6%), p-Cymene (6.27%), Thymol (4.10), γ-Terpinene (3.97) and Linalool (2.53%) which are known to have toxic activity against stored product insect [37]. The proportion of main components obtained in *L. origanoides* oil used in this experiment, was higher in the percentage in which Carvacrol compound appeared, and lower in the percentage in which the rest of the components appeared to those described by Alcala-Orozco [38].

The genus *Lippia* spp., belonging to the family Verbenaceae, has been investigated with regard to its insecticidal properties against other insect storage pests, as for example, *Tribolium castaneum* [39]. The main compounds for *L. alba* were the terpenoid Carvone (25.17%), the monoterpenoids β-Myrcene (12.00%), Geranial (11.87%), Neral (10.50%) and the terpene Limonene (9.70%). That is, the main essential oil components of *L. alba* oil are the monoterpenes. Monoterpenes possess insecticidal and insect repellent properties as described by Isman [40], Silva et al. [41] and Chen and Viljoen [42].

Martins et al. [43] and Vieira and Simon [44] described that the chemical composition of essential oils can show large variability, both interspecific and within the same species. It seems to depend on the genetic characteristics of the plant and on the conditions under which it was grown. This has been observed when the components of the essential oils of different *L. alba* plants grown in different areas of Brazil were analyzed [33].

These essential oils evaluated of *L. origanoides* and *L. alba* exhibited similar patterns for insecticidal activity over the *A. obtectus* when sprayed directly in Petri and accumulated an insect mortality of 85.00 and 81.94%, respectively, significantly greatest than the lower applied doses of each essential oil. The insecticidal activity of both oils did not exceed 15 days after application. In the same range, it is well described by Ilboudo et al. [45] for several other essential oils. Losses of activity for essential oils are normally due to degradation of the active compounds. Essential oils containing more hydrogenated compounds are more susceptible to oxidation [46]. Various studies with essential oils obtained from species of the genus *Lippia* spp., as for example *L. javanica* also showed good results regarding their insecticidal effect against other insect pests [47].

Other studies have shown diverse activities and effects of *L. origanoides* against insect storage pests with it main component (Carvacrol), that exhibited the maximum percentage of mortality and the highest repellent potency with RC_{50} of 0.22% for *T. castaneum*, and 0.20% for *Ulomoides dermestoides* [38]. While, Rozman et al. [48] reported toxicity of fumigated linalool against *T. castaneum* Herbst (Coleoptera: Tenebrionidae), *Rhyzopertha dominica* (Coleoptera: Bostrichidae), and *Sitophilus oryzae* (Coleoptera: Curculionidae), in fumigation with Linalool, one of the compounds of *L. origanoides* essential oil, where Linalool was highly effective for *R. dominica*, and caused 100% mortality at the lowest tested concentration (0.1 mL/720 mL of volume).

The limonene compound present in the essential oil of *L. alba* has toxic fumigant activity against *T. castaneum* [49] (LEE et al., 2003). Caballero-Gallardo et al. [50] have shown that substances such as benzylbenzoate, β-myrcene and carvone, also present in the essential oil of *L. alba*, have good repellent properties against this pest. Tripathi et al. [51,52] also demonstrated the toxic effect of d-limonene and carvones on *T. castaneum* larvae and adults, by contact and by fumigation.

The results prove the insecticidal capacity of essential oils of *Lippia* spp. genus and hence their potential as active substances against *A. obtectus.* Joining in this way, the numerous genera of plants known and whose essential oils have shown insecticidal activity against this insect [53,54], in addition to other recently discovered biological control agents, such as fungi [55–58] or bacteria [59].

5. Conclusions

L. origanoides and *L. alba* essential oils exhibited similar patterns of insecticidal activity over the insect. *L. origanoides* and *L. alba* accumulated an insect mortality of 85.00 and 81.94%, respectively, significantly greatest than the lower applied doses of each essential oil. However, all the lower doses applied of each oil were significantly greatest than the control treatment, with an accumulated mortality of 16.25%. These essential oils affected the survival of *A. obtectus* since the greatest doses applied on insects decreased the life of the bean weevil. The results prove the insecticidal capacity of essential oils of *Lippia* spp. genus and hence their potential as active substances against *A. obtectus* in environmentally low risk pest control strategies. Supplementary trials should be conducted under real storage conditions.

Author Contributions: A.R.G. and F.D.S. designed the experiment. A.R.G. and P.A.C. conducted the experiments. P.A.C performed statistical analysis. A.R.G, F.D.S. and P.A.C prepared the manuscript. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest: The authors declare no conflict of interest.

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